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Corroding iron as a hydrogen source for sulphate reduction in growing cultures of sulphate-reducing bacteria

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Summary. In anaerobic corrosion experiments, hydrogenase-positive *Desulfovibrio* strains, grown with limiting lactate concentrations in the presence of steel wool, formed more sulphide than expected or observed with lactate alone. The additional sulphide obviously originated from sulphate reduction with cathodically formed hydrogen from the steel surface. The hydrogenase-negative *D. sapovorans* did not produce additional sulphide. The observations agree with the theory of von Wolzogen Kühr and van der Vlugt (1934) that explains anaerobic corrosion as a cathodic depolarization of iron surfaces by hydrogen-consuming sulphate-reducing bacteria. The influence of the iron surface area, the salt concentration and the pH-value on the utilization of cathodically formed hydrogen was investigated. The significance of an additional organic electron donor for the corrosion of iron in aqueous environments is discussed.

Introduction

The anaerobic corrosion of iron is of great economic importance (Postgate 1984). It diminishes drastically the life time of steel and iron material (e.g.: offshore oil-pipes, sewage pipes, oiltanks) in aqueous, oxygen-free, reduced environments (e.g.: marine sediments, interior of sewage pipes, flooded soils (gley)). The rapidity of metal decay is mostly due to locally corroded areas (pitting corrosion).

When iron is immersed in water, it releases Fe^{++}-cations, whereas the metal surface becomes negatively charged by the remaining electrons (Table 1, Eq. 1). The dissolving process continues only if the electrons are removed, e.g. by an oxidizing agent. Under aerobic conditions oxygen serves as electron acceptor. The result is rust-formation.

In the absence of oxygen, the electrons left on the metal-surface reduce protons, from the dissociation of water, to hydrogen (Eq. 2, 3) which remains on the iron surface and protects the iron from further dissolving. A dynamic equilibrium is established which keeps the iron polarized. The classical theory of von Wolzogen Kühr and van der Vlugt (1934) suggests that the principal mechanism of anaerobic corrosion is a cathodic depolarization of the iron surface by hydrogen-consuming sulphate-reducing bacteria (SRB). The proposed reactions are listed in Table 1. SRB are supposed to disturb the equilibrium by oxidation of the cathodically formed elemental (or atomic) hydrogen (briefly termed "cathodic hydrogen"), with sulphate as electron acceptor (cathodic depolarisation). The result is a net oxidation of the metal.

In an amended corrosion theory it was suggested that solid ferrous sulphide in contact with iron acts as a cathode, like a noble metal, and facilitates the depolarization (Booth et al. 1968). Other authors proposed that reduced phosphorous compounds, too, are involved in the anaerobic corrosion process (Iverson and Olson 1984).

Anaerobic corrosion was usually measured as weight-loss of iron specimens (Booth and Wormwell 1961; Booth et al. 1966, 1967, 1968; Bell and Chor Kiang Lim 1981; King et al. 1973) or indirectly by changes in current/potential curves (polarization curves; Booth et al. 1968; Hardy 1983). Hardy (1983) supported the depolarization-theory

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by demonstrating that concentrated cell suspensions of SRB reduced labelled sulfate in the presence of an iron-working electrode as sole hydrogen donor.

If corroding iron really serves as a hydrogen source for hydrogenase-positive SRB, it should be possible to investigate bacterial corrosion by determining the sulphide formed from the oxidation of cathodic hydrogen. It was the aim of the present work to quantify the anaerobic corrosion of iron under almost natural conditions (without application of an external electron-motive-force, without artificial concentration of bacterial cell suspensions) by measuring the amount of cathodic hydrogen oxidized by growing cultures of SRB.

Material and methods

Organisms. Desulfovibrio vulgaris strain Marburg (DSM 2119) was kindly provided by Prof. R. K. Thauer, Universität Marburg. *Desulfovibrio sapovorans* strain Lindhorst (DSM 2055) has been subcultured since its isolation (Pfennig et al. 1981). The salt requiring *Desulfovibrio* strains LaViS and LaVit were isolated from water of an oilfield in Northern Germany (Cord-Ruwisch et al. 1985).

Media and growth conditions. All cultures of sulphate-reducing bacteria (SRB) were grown in defined bicarbonate-buffered, sulphide-reduced media containing 20 mM of sulphate, as described previously (Pfennig et al. 1981; Widdel and Pfennig 1984). The medium for the *Desulfovibrio* strains LaViS and LaVit contained 30 g NaCl/l. If not otherwise noted, lactate (10 mM) served as energy and carbon source. *Desulfovibrio* species able to utilize hydrogen have active hydrogenase, also if grown on lactate (Postgate 1984).

Corrosion experiments. Steel wool (RAKSO, Germany), with a surface area of about 100 cm^2/g, served as the source of metallic iron. (The surface area was calculated from the weight of a specimen of 100 mm × 0.5 mm × approx. 0.013 mm: 5.1 mg) 0.5 g of the steel wool was surface-activated for about 5 min in diluted HCl (2 M) until substantial formation of hydrogen bubbles occurred. The steel wool was immediately rinsed in sterile distilled water for 10 s and added to the reduced culture medium in 20 ml Hungate-tubes that were completely filled. The tubes were sealed with butyl-rubber septa and inoculated with sterile synringes by replacing 5% of the culture liquid by freshly grown cultures of SRB. The rubber septum served as a pressure buffer. Cultures were incubated for 14 days at 37° C if not otherwise stated. Thereafter, the sulphide dissolved and precipitated was determined by use of the previously described method based on formation of colloidal copper sulphide (Cord-Ruwisch 1985).

Stoichiometric calculations. All strains used were incompletely oxidizing SRB, which produce 1 mol sulphide per 2 mol lactate oxidized:

$$2\,CH_3—CHOH—COO^- + SO_4^{2-} \longrightarrow 2\,CH_3—COO^- + H_2S + 2\,HCO_3^-$$

Hence, 10 mM lactate would yield 5 mM sulphide. Formation of more sulphide by hydrogen-consuming SRB in the presence of steelwool was explained as the product of bacterial sulphate reduction with cathodic hydrogen from the metal surface. According to the cathodic depolarization theory of von Wolzogen Kühr and van der Vlugt (1934), 1 mol sulphide produced from cathodic hydrogen corresponds to 4 mol Fe oxidized to Fe^{2+} (Table 1, Eq. 7). In our experiments (25 dm^2 iron surface area/l) 1 mmol sulphide per litre and day produced from cathodic hydrogen corresponded to approx. 8.93 mg iron corroded per dm^2 of surface area and day.

Results

Importance of the organic electron donor

Steel wool did not allow sulphide-production by hydrogenase-positive *Desulfovibrio* species (in the presence of 1 mM acetate as carbon source), unless a favourable organic energy source such as lactate was present. With additional limiting amounts of lactate, however, the hydrogenase-positive *Desulfovibrio vulgaris* produced more sulphide in the presence of metallic iron than was possible from lactate-oxidation alone (Table 2). The hydrogenase-negative *Desulfovibrio sapovorans* did not produce additional sulphide. The sulphide produced in excess of that from lactate oxidation can, therefore, only be explained as a result of the utilization of molecular hydrogen

Table 1. Reactions during anaerobic (corrosion by sulphate reducers as proposed by the depolarization theory of von Wolzogen Kühr and van der Vlugt (1934)

eq. 1	Anodic reaction:	$4\,Fe$	$\longrightarrow 4\,Fe^{2+} + 8\,e^-$
eq. 2	Water dissociation:	$8\,H_2O$	$\longrightarrow 8\,H^+ + 8\,OH^-$
eq. 3	Cathodic reaction:	$8\,H^+ + 8\,e^-$	$\longrightarrow 8\,H \longrightarrow 4\,H_2$
eq. 4	Hydrogen oxidation:	$SO_4^{2-} + 4\,H_2$	$\longrightarrow H_2S + 2\,H_2O + 2\,OH^-$
eq. 5	Sulfide precipitation:	$Fe^{2+} + H_2S$	$\longrightarrow FeS \;\; 2\,H^+$
eq. 6	Hydroxide formation:	$3\,Fe^{2+} + 6\,OH^-$	$\longrightarrow 3\,Fe(OH)_2$
eq. 7	Overall reaction:	$4\,Fe + SO_4^{2-} + 4\,H_2O$	$\longrightarrow FeS + 3\,Fe(OH)_2 + 2\,OH^-$

Table 2. Sulphide production by *Desulfovibrio sapovorans* (hydrogenase negative) and *D. vulgaris* (hydrogenase positive) after 14 days in freshwater medium with steel wool and/or lactate

Lactate given (mM)	Sulphide (mM)					
	Theoretically possible from lactate oxidation[a]	Produced by *D. sapovorans*[b]		*D. vulgaris*[b]		
		−steel wool	+steel wool	−steel wool	+steel wool	from cath. H_2
0	0	0	0	0	0[c]	0
10	5.0	3.6	3.9	4.0	6.6	1.6
15	7.5	6.2	6.9	6.5	10.1	2.6

[a] Calculated from the equation $2\,CH_3—CHOH—COO^- + SO_4^{2-} \leftarrow - - - \rightarrow 2\,CH_3COO^- + 2\,HCO_3^- + H_2S$

[b] The amount of sulfide determined in the control assays without lactate and without steel wool has been subtracted from all values measured

[c] Also tested after 54 days of incubation

formed on the iron surface. In assays with hydrogenase-positive strains, the FeS produced adhered as a dense black layer to the iron surface. In contrast, in experiments with the hydrogenase-negative *D. sapovorans*, the steel wool kept its greyish surface. In the presence of lactate plus iron the hydrogenase positive strains were still motile after 14 days of incubation. In the absence of iron with lactate alone, most of the cells were lysed after this period and motile cells were not detected.

The sulphide production from cathodic hydrogen in the presence of different lactate concentrations was also tested with a salt-requiring hydrogenase-positive *Desulfovibrio* isolate (strain LaViS) from an oilfield (Table 3). The amount of cathodic hydrogen removed from the iron surface was the same for both hydrogenophilic strains (*D. vulgaris* and strain LaViS) and nearly proportional to the concentration of the added lactate. Between 24% and 33% of the electrons for the sulphate reduction was derived from the iron (Tables 2, 3).

Table 3. Sulphide production by *Desulfovibrio* strain LaViS with cathodic hydrogen from steel wool and different concentrations of lactate[a]

Lactate given (mM)	Sulphide (mM)		
	Theoretically possible from lactate oxidation	Totally produced	From oxidation of cathodic hydrogen
5	2.5	4.1	1.6
10	5.0	7.7	2.7
20	10.0	13.3	3.3
40[a]	20.0	26.4	6.4

[a] Sulphate concentration was 40 mM

Influence of the iron surface area

The influence of the surface area of the steel wool on sulphide formation with cathodic hydrogen was tested by adding different amounts of steel wool to the tubes (Table 4). In the presence of 10 mM lactate, significant oxidation of cathodic hydrogen occurred if the surface area of the steel wool was larger than 10 dm^2/l of culture medium. A surface area larger than 25 dm^2/l did not further increase the amounts of sulphide after 14 days of incubation in the presence of 10 mM lactate.

Time course of cathodic hydrogen consumption

In order to study the time course of anaerobic corrosion, a series of tubes containing lactate medium and steel wool was inoculated with *Desulfovibrio* strain LaViS. After different periods of in-

Table 4. Influence of the iron surface area on sulphide production by *Desulfovibrio* strain LaViS in the presence of 10 mM lactate

Iron surface (dm^2/l)	Sulphide produced (mM)		
	Dissolved[a]	Total	From cathodic hydrogen
0	5.1	5.1	—
1	5.1	5.1	—
5	1.2	4.7	—
10	0.0	5.6	0.5
25	0.0	5.6	2.7
50	0.0	7.8	2.7
100	0.0	7.7	2.6

[a] Determined before dissolving FeS by acid

cubation, the totally formed sulphide was determined in two tubes from the series (Fig. 1). Oxidation of cathodic hydrogen was fastest during the first 3 days, i.e. when lactate was probably still present. During this time the *Desulfovibrio* strains LaViS produced 0.83 mM sulphide/day by the oxidation of cathodic hydrogen. This corresponds to a corrosion-rate of at least 7.4 mg Fe/dm^2 · day. After about 3 days, the corrosion continued only slowly with a rate of approximately 1.8 mg/dm^2 · day.

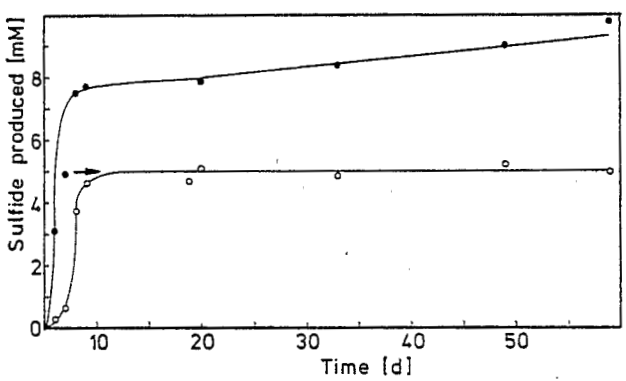

Fig. 1. Time course of total sulphide production by *Desulfovibrio* strain LaViS in medium with lactate (10 mM) in the presence (●---●) or absence (○---○) of steel wool. The arrow indicates the maximum sulfide concentration that can be theoretically formed from the added lactate concentration. (Further information is given in the text)

Influence of pH, salinity and temperature

Low pH-values, which favour aerobic corrosion, are expected to stimulate also the anaerobic corrosion by shifting the proton-hydrogen-equilibrium on the polarized metal-surface in the direction of hydrogen formation. However, strain LaViS utilized cathodic hydrogen fastest at a pH of 7 which was also optimal for its growth (Fig. 2).

Also changing of salt concentrations between 1 and 100 g NaCl per l did not increase the amount of oxidized cathodic hydrogen (Fig. 3). Strain LaViS was most "corrosive" at its optimal salt concentrations of about 30 g/l.

The influence of the temperature on bacterial cathodic hydrogen oxidation was studied using *Desulfovibrio* strain LaViT, which tolerated temperatures up to 49 °C and which had an optimum of 43 °C. At 34 °C this strain consumed less cathodic hydrogen within 14 days than strain LaViS. At higher temperatures, however, the amount of cathodic hydrogen oxidized by strain LaViT and thus the corrosion-rate increased (Fig. 4). The increase was still observed somewhat above the optimum temperature of growth. At 46 °C strain LaViT oxidized more cathodic hydrogen (13.2 mM per 14 days) than strain LaViS at 34 °C (11.2 mM).

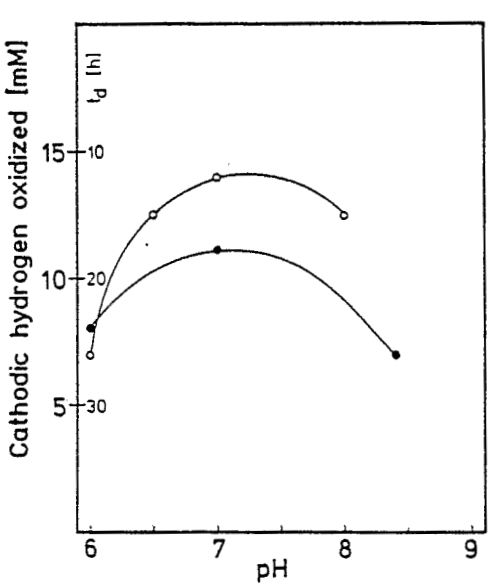

Fig. 2. Influence of the pH-value on cathodic hydrogen utilization by *Desulfovibrio* strain LaViS. ○---○ = doubling time (t_d) on lactate only; ●---● = cathodic hydrogen oxidized

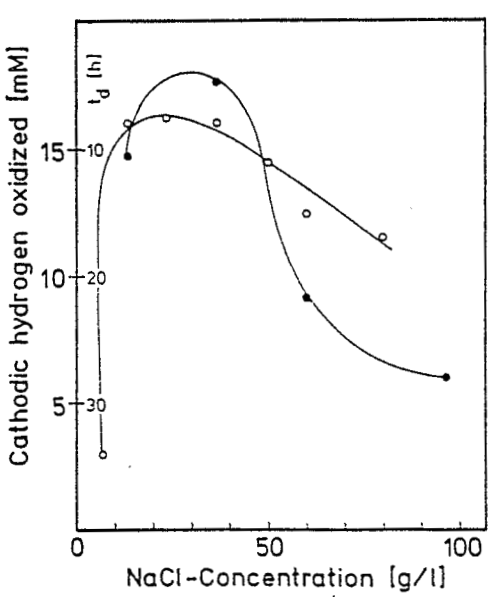

Fig. 3. Influence of the NaCl-concentration on the cathodic hydrogen-utilization by *Desulfovibrio* strain LaViS. ○---○ = doubling time on lactate only; ●---● = cathodic hydrogen oxidized

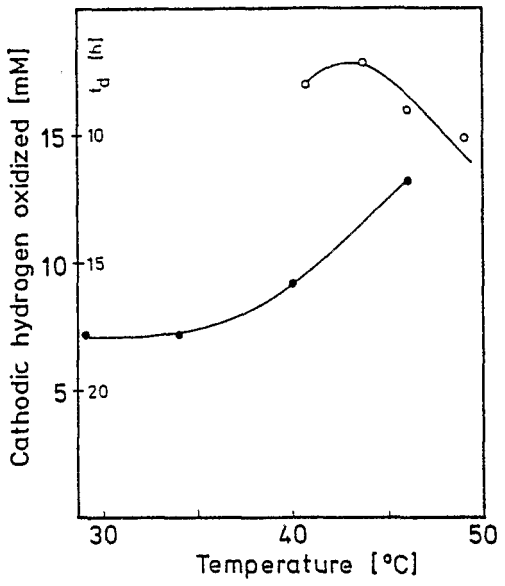

Fig. 4. Influence of the temperature on cathodic hydrogen-utilization by *Desulfovibrio* strain LaViT. O---O = doubling time on lactate only; ●---● = cathodic hydrogen oxidized

Discussion

The use of steel wool providing a large surface area of metallic iron (approx. 25 dm^2/l of medium) and the determination of sulphide formed instead of the metal weight loss, turned out to be useful methods for investigating the involvement of sulphate reducers in anaerobic corrosion. The experiments demonstrate that metallic iron without application of an external electron-motive-force is in fact used as a source of reducing equivalents for dissimilatory reduction of sulphate in growing cultures of hydrogenase-positive SRB. The reducing equivalents were obviously molecular hydrogen formed by the cathodic reaction of iron with protons from water (Table 1, Eq. 3). The observations confirm the classical theory of von Wolzogen Kühr and van der Vlugt (1934).

However, *Desulfovibrio* used cathodically formed hydrogen only if an organic electron donor such as lactate was present. Iron alone did not allow sulphate-reduction during the period of the experiment (up to 54 days). To explain the dependence of the cathodic hydrogen oxidation on the presence and concentration of an organic electron donor, two, possibly cumulative effects have to be considered:

Firstly, the cathodic hydrogen was preferentially oxidized together with the organic substrate (Fig. 1). The possibility of simultaneous utilization of hydrogen and an organic substrate, ethanol, has been demonstrated with *Desulfobulbus propionicus* (Laanbroek et al. 1982).

Secondly, sulphide from sulphate reduction with the organic substrate (lactate) reacts with remaining ferrous ions or ferrous hydroxide from the anodic process (Table 1, Eq. 5, 6) to further ferrous sulphide. The scavenging of ferrous iron as almost insoluble sulphide may promote ("pull") the overall reaction (Table 1, Eq. 7) significantly (anodic depolarization). Furthermore, the increase of the precipitated ferrous sulphide may also increase its effectivity as cathode (Booth et al. 1968), especially if the FeS remains firmly attached to the metallic surface as observed in our experiments with hydrogenase-positive SRB.

The first effect (simultaneous oxidation of H_2 and lactate) was apparently the more important one in short-term H_2-removal from the iron surface. The utilization of cathodic hydrogen was faster during the oxidation of organic substrate than after its depletion. The corrosion-rate diminished from 7.4 mg/dm^2·day initially, to 1.8 mg/dm^2·day after consumption of lactate in spite of increased sulfide concentrations (Fig. 1).

The corrosion rates obtained with the described method are in the same order of magnitude as those observed by weightloss measurements in batch cultures by other authors (Booth and Wormwell 1961). However, 10 to 20 fold higher corrosion rates are observed in continuous culture experiments (Booth et al. 1966, 1967; Bell and Chor Kiang Lim 1981).

In conclusion, the availability of organic electron donors appears to be an important factor that influences the removal of cathodic hydrogen from iron surfaces. Hence, anoxic aqueous environments rich in anaerobically degradable organic matter (e.g. interior of sewage pipes) should be more corrosive than environments that are mainly inorganic (e.g. water in geothermal heating plants).

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